

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization  
International Bureau



(43) International Publication Date  
11 December 2003 (11.12.2003)

PCT

(10) International Publication Number  
**WO 03/101477 A1**

- (51) International Patent Classification<sup>7</sup>: **A61K 38/17, A61P 33/02**
- (74) Agents: **MINOJA, Fabrizio et al.**; Bianchetti Bracco Minoja S.r.l., Via Rossini, 8, I-20122 Milano (IT).
- (21) International Application Number: **PCT/EP03/05551**
- (81) Designated States (*national*): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NI, NO, NZ, OM, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.
- (22) International Filing Date: **27 May 2003 (27.05.2003)**
- (25) Filing Language: **English**
- (26) Publication Language: **English**
- (30) Priority Data:  
**MI2002A001205** **4 June 2002 (04.06.2002)** **IT**
- (84) Designated States (*regional*): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE, SI, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).
- (71) Applicant (*for all designated States except US*): **PHARMAPRODUCTS UK LIMITED** [GB/GB]; 7th Floor, Castle Chambers, 43 Castle Street, Liverpool, Merseyside L2 9TL (GB).
- (72) Inventors; and
- (75) Inventors/Applicants (*for US only*): **SALVAGGIO, Antonio** [IT/IT]; Via Cesare Battisti, 35, I-95021 Acicastello (CT) (IT). **NICOLETTI, Pierferdinando** [IT/IT]; Via Luigi Sturzo, 3, I-95021 Cannizzaro (CT) (IT). **MACRI', Battesimo** [IT/IT]; Polo Universitario dell'Annunziata, c. da SS Annunziata, I-98168 Messina (IT).
- Published:**  
— *with international search report*
- For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.*

(54) Title: **USE OF UK114 IN THE TREATMENT OF LEISHMANIASIS**

(57) Abstract: This invention relates to the use of the protein UK114, possibly associated with ubiquitin, for the treatment of leishmaniasis in humans and animals.

**WO 03/101477 A1**

## USE OF UK114 IN THE TREATMENT OF LEISHMANIASIS

This invention relates to the use of the protein UK114, possibly associated with ubiquitin, to treat leishmaniasis in humans and animals.

The protozoa of the *Leishmania* genus are intracellular parasites of the macrophages and dendritic cells of the dog, man and numerous wild animals.

5 On the basis of the classification criteria used in human medicine, leishmaniasis presents in three clinical forms: visceral (known in man as "kala-azar"), cutaneous and mucocutaneous.

In the case of the dog, a cutaneous and a visceral form were separately classified in the past because of the characteristic clinical picture, but they are  
10 now both regarded as progressive forms of the same disease, known as "generalised canine leishmaniasis".

The vector is a sand-fly of the *Phlebotomus* genus in the Old World and the *Lutzomyia* genus in the New World. The protozoa multiply in the sand-fly and are transformed into infectious organisms.

15 Parasites of the *Leishmania* genus appear as rounded or oval organisms in the macrophage, with the rod-like kinetoplast adjacent to the nucleus. The organism, which measures 2 to 5  $\mu\text{m}$  in diameter, possesses a rudimentary flagellum that does not extend beyond the edge of the cell. This amastigote form of the parasite is ingested by the sand-fly during the blood meal. The  
20 protozoon is transformed in the intestine of the intermediate host into the promastigote form, characterised by a long free flagellum that protrudes from the anterior extremity of the parasite. The organism has an elongated shape and can grow to a length of 15  $\mu\text{m}$ , excluding the flagellum, which usually has the same dimensions as the body.

25 The amastigotes ingested reach the intestine of the sand-fly, where they are transformed into promastigotes. The promastigotes divide repeatedly by

binary fission, and subsequently migrate in the anterior direction. In the pharynx, the parasites turn into highly mobile metacyclic promastigotes, which migrate towards the proboscis. The promastigotes are transmitted to the new vertebrate host by means of the sand-fly's bite.

5 In the vertebrate host, the promastigotes are ingested by the monocytes/macrophages. After being ingested, the promastigote turns into an amastigote. The amastigotes divide by binary fission in the parasitophorous vacuole until their number is sufficient to rupture the macrophage. The amastigotes thus released are ingested by other macrophages.

10 The ability of the amastigotes to survive in the macrophages and spread throughout the body depends on factors intrinsic to the parasite and on factors associated with the type of cell-mediated immune response developed by the host. If parasitic macrophages are sufficiently stimulated by T-helper (Th) lymphocytes, they produce numerous lysosome enzymes and other factors  
15 including oxygen metabolites, hydrogen superoxide and peroxide and nitrous oxide (NO), which are toxic to the parasite.

The type of cell-mediated immune response and interleukin (IL) profile produced determine resistance or sensitivity to *Leishmania* infection. In laboratory animals, resistance to *Leishmania* infection is characterised by the  
20 Th1 response, with production of IL-12 and interferon gamma (IFN $\gamma$ ) and activation of the macrophages which eliminate the parasite. Conversely, in animals sensitive to infection, the response is type Th2, characterised by production of IL-4 and IL-10 with consequent suppression of the parasitocidal activity of the macrophages and stimulation of the B lymphocytes with an  
25 increase in the production of immunoglobulins.

The humoral immune response in leishmaniasis is impressive, but not protective. The specific antibodies produced against *Leishmania* have no neutralising action against the parasite.

In animals sensitive to the disease the protozoon spreads throughout the body, in the macrophages. The parasite has been observed in all the organs and tissues of the body except the central nervous system. Slow, continuous contact between the parasitic antigen and the immunocompetent cells forms the basis for the pathogenetic development of the disease, which is characterised by:

hyperglobulinaemia, generally polyclonal, associated with continual stimulation of the B lymphocytes, which causes an increase in total proteins and inversion of the albumin/globulin ratio;

production of auto-antibodies, probably due to a cross-reaction between parasitic antigens and self-antigens, causing thrombocytopenia and anaemia;

production and deposit of immunocomplexes responsible for the vasculitis, glomerulonephritis and polyarthritis syndromes.

The pathogenesis of the skin lesions present in most sufferers is not yet clear. According to some authors, the persistence of the parasite in the macrophage continually stimulates infiltration by inflammatory cells, especially plasma cells, macrophages and lymphocytes, into the dermis. According to other authors, the deposit of immunocomplexes is the main cause of dermatitis which, on histological examination, often presents lesions similar to those caused by other diseases induced by immunocomplexes, such as systemic Lupus erythematosus. Finally, the skin alterations may be the result of vasculitis.

The symptoms of canine leishmaniasis are highly variable, and may include peripheral lymphadenopathy (over 90% of infected subjects), skin lesions (>80%), chronic conjunctivitis (50%), onychogryphosis (40%), anorexia (>35%), increased appetite (30%), weight loss (30%), fever (20%), kidney failure (20%), epistaxis (10%), uveitis (8%) and gait disorders (6%).

The skin signs are among the most important in the disease. Various

types of macroscopic and microscopic lesions have been described in canine leishmaniasis: dry exfoliative dermatitis, ulcerative dermatitis, nodular dermatitis, sterile pustular dermatitis, paronychia, and nasal and/or digital hyperkeratosis. The skin lesions are generally chronic, symmetrical and not itchy.

Recent studies clearly demonstrate the existence of a TNF-independent compensation mechanism able to activate the macrophages in the anti-leishmania response. As the Th1 response mediated by interleukin-12 (IL-12) and interferon gamma (IFN $\gamma$ ) is paradoxically responsible not only for activation of the macrophages, but also for nearly all the symptoms of leishmaniasis, it may be advantageous to boost this compensation mechanism by inhibiting the Th1 response.

The current elective treatment, based on antimony gluconate administered by infiltration (in cutaneous l.) or injection (in the other forms) can cause toxic effects (nausea and vomiting) sufficiently serious to require discontinuance of the treatment, which is replaced by treatment with aromatic diamines such as pentamidine, whose tolerability is generally poor.

It has now been found that the protein with molecular weight 14 kDa in SDS-PAGE, obtainable by extraction from mammal liver with perchloric acid, called UK114 and disclosed in EP 574394 and US 5792744, is useful in the treatment of leishmaniasis, possibly associated with ubiquitin (UK110).

Recombinant protein UK114 is known from WO 00/63368.

Subcutaneous administration of UK114 and ubiquitin to a group of 10 dogs with manifest clinical symptoms of leishmaniasis (peripheral lymphadenopathy and skin lesions of a high degree, mainly represented by sores and bleeding ulcers with loss of substance, anorexia and weight loss), at the doses and times indicated in the table, led to complete healing of all the lesions during the treatment period.

No adverse effects were observed during the treatment.

These findings demonstrate that the administration of UK114 and ubiquitin cures the clinical symptoms of leishmaniasis in a totally safe manner. This is very interesting in view of the high toxicity of the drugs currently used in treatment, and the possibility of a response that is sometimes unsatisfactory from the clinical standpoint.

TABLE

Patient's weight < 10 kg	1mg/day subcutaneously for 6 days 7th day: rest 1 mg/day subcutaneously for 6 more days
Patient's weight > 10 kg	As above, but doubling the dose: 2 mg/day

According to the invention, protein UK114 of extractive or recombinant origin will therefore be opportunely administered to subjects suffering from leishmaniasis by the parenteral route, in particular subcutaneously or intramuscularly, at doses ranging between approx. 0.5 and 10 mg a day, until the disappearance or substantial reduction of the symptoms. The compositions according to the invention, in the form of solutions or suspensions in preferably aqueous sterile solvents, may also contain ubiquitin in a quantity corresponding to 0.1-5 mg per unit dose.

**BIBLIOGRAPHY**

1. **Urquhart G.M., Armour J., Duncan J.L., Dunn A.M, Jennings F.W.**  
Veterinary Parasitology 2<sup>nd</sup> ed., Italian edition edited by C. Genchi, UTET  
5 1998.
2. **Murray H.W., Jungbluth A., Ritter E., Montelibano C., Marino  
M.W.** Visceral Leishmaniasis in Mice Devoid of Tumor Necrosis Factor and  
Response to Treatment, Infection and Immunity, November 2000, p. 6289-  
6293, Vol. 68, No. 11.
- 10 3. **Katzung B.G.** Basic & Clinical Pharmacology, 1995 by Appleton &  
Lange, E.N., Connecticut.

**CLAIMS**

1. Use of the protein UK114, possibly in combination with ubiquitin, for the preparation of pharmaceutical or veterinary compositions for the treatment  
5 of leishmaniasis in humans and animals.
2. Pharmaceutical or veterinary compositions containing the extractive or recombinant protein UK114, possibly associated with ubiquitin, together with a suitable vehicle, for the treatment of leishmaniasis in humans and animals.

## INTERNATIONAL SEARCH REPORT

International Application No  
PCT/EP 03/05551

A. CLASSIFICATION OF SUBJECT MATTER  
IPC 7 A61K38/17 A61P33/02

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, WPI Data, PAJ, BIOSIS, EMBASE

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 98 42366 A (MERONI PIER LUIGI ;ZETESIS SPA (IT); PANERAL ALBERTO (IT); BARTORE) 1 October 1998 (1998-10-01)	2
A	page 1, line 20 - line 24 page 2, line 11 - line 16 page 4, line 1 - line 5 ----- -/-	1

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

## \* Special categories of cited documents :

\*A\* document defining the general state of the art which is not considered to be of particular relevance

\*E\* earlier document but published on or after the international filing date

\*L\* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

\*O\* document referring to an oral disclosure, use, exhibition or other means

\*P\* document published prior to the international filing date but later than the priority date claimed

\*T\* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

\*X\* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

\*Y\* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

\*G\* document member of the same patent family

Date of the actual completion of the international search

9 September 2003

Date of mailing of the international search report

26/09/2003

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2  
NL - 2280 HV Rijswijk  
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,  
Fax: (+31-70) 340-3016

Authorized officer

Hars, J

## INTERNATIONAL SEARCH REPORT

International Application No

PCT/EP 03/05551

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with Indication, where appropriate, of the relevant passages	Relevant to claim No.
X	PANERAL A E ET AL: "CHRONIC ADMINISTRATION OF UK-114, A MULTIFUNCTIONAL EMERGING PROTEIN, MODULATES THE TH1/TH2 CYTOKINE PATTERN AND EXPERIMENTAL AUTOIMMUNE DISEASES" ANNALS OF THE NEW YORK ACADEMY OF SCIENCES, NEW YORK ACADEMY OF SCIENCES, NEW YORK, NY, US, vol. 876, 1999, pages 229-235, XP000971426 ISSN: 0077-8923	2
A	abstract page 230, paragraph 1 - paragraph 2 page 233, paragraph 1 page 234, last paragraph	1
X	NICOLETTI FERDINANDO ET AL: "Prevention and treatment of lethal murine endotoxemia by the novel immunomodulatory agent MFP-14." ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, vol. 45, no. 5, May 2001 (2001-05), pages 1591-1594, XP002253826 ISSN: 0066-4804	2
A	abstract page 1591, left-hand column -right-hand column, paragraph 2	1
X	WO 99 43340 A (ZETESIS SPA ;SANTI CESARE (IT); BARTORELLI ALBERTO (IT)) 2 September 1999 (1999-09-02) page 1 claim 4	2
X	WO 00 78329 A (ZETESIS SPA ;PANERAI ALBERTO (IT); BARTORELLI ALBERTO (IT); NICOLE) 28 December 2000 (2000-12-28) claim 1	2
A	DATABASE BIOSIS 'Online! BIOSCIENCES INFORMATION SERVICE, PHILADELPHIA, PA, US; July 2001 (2001-07) KHASKHELY NOOR MOHAMMAD ET AL: "Pre-exposure with low-dose UVA suppresses lesion development and enhances Th1 response in BALB/c mice infected with Leishmania (Leishmania) amazonensis." Database accession no. PREV200100345475 XP002253827 abstract & JOURNAL OF DERMATOLOGICAL SCIENCE, vol. 26, no. 3, July 2001 (2001-07), pages 217-232, ISSN: 0923-1811	1,2

## INTERNATIONAL SEARCH REPORT

International Application No  
PCT/EP 03/05551

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9842366	A	01-10-1998	IT MI970694 A1 25-09-1998
			AU 7207298 A 20-10-1998
			BR 9808625 A 16-05-2000
			CN 1251043 T 19-04-2000
			WO 9842366 A1 01-10-1998
			EP 0969859 A1 12-01-2000
			HU 0002304 A2 28-11-2000
			JP 2001518107 T 09-10-2001
			NO 994622 A 23-09-1999
			NZ 337985 A 23-02-2001
			PL 335829 A1 22-05-2000
			TR 9902331 T2 21-01-2000
			US 6255283 B1 03-07-2001
WO 9943340	A	02-09-1999	IT MI980356 A1 24-08-1999
			AT 208627 T 15-11-2001
			AU 2624499 A 15-09-1999
			CA 2321717 A1 02-09-1999
			DE 69900466 D1 20-12-2001
			DE 69900466 T2 04-04-2002
			DK 1061938 T3 11-02-2002
			WO 9943340 A1 02-09-1999
			EP 1061938 A1 27-12-2000
			ES 2165727 T3 16-03-2002
			JP 2002504517 T 12-02-2002
			PT 1061938 T 29-04-2002
WO 0078329	A	28-12-2000	IT MI991384 A1 22-12-2000
			AU 5221700 A 09-01-2001
			BR 0011832 A 05-03-2002
			CA 2377639 A1 28-12-2000
			CN 1399555 T 26-02-2003
			CZ 20014631 A3 17-04-2002
			WO 0078329 A2 28-12-2000
			EP 1194158 A2 10-04-2002
			HU 0201796 A2 28-09-2002
			JP 2003502380 T 21-01-2003
			NO 20016326 A 21-12-2001
			NZ 516159 A 25-07-2003
			TR 200103748 T2 21-05-2002
			ZA 200110213 A 12-12-2002